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PROTEINS

Cast Films from Soy Protein Isolates and Fractions

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ABSTRACT

Glycerol-plasticized soy protein films were cast from alkaline aqueous film-forming solutions of laboratory-prepared 7S, 11S, and soy isolate (LSI) fractions and from commercial soy isolate (CSI). Tensile strength (TS), elongation at break (E), water vapor permeability (WVP), total soluble matter (TSM), protein solubility (PS), and Hunter L, a, and b color values of these films were determined. The 11S films had greater TS than 7S films (\( P < 0.05 \)), while LSI films had greater TS than CSI films (\( P < 0.05 \)). No significant differences were detected among mean E values and among mean WVP values of all films (\( P > 0.05 \)). The 7S films had higher TSM and PS values than 11S films (\( P < 0.05 \)). CSI films were significantly darker (lower L value) and more yellow (greater positive b value) than LSI films (\( P < 0.05 \)).

Edible films and coatings can prolong the shelf life of foods due to their selective barrier properties against movement of moisture, gases, and vapor. Interest in edible packaging is likely to grow in the near future as it has potential for reducing the use of nonedible or synthetic packaging (Gennadios et al. 1993a). Kester and Fenfema (1986) discussed edible films derived from several polysaccharides, lipids, and proteins. Formation and properties of films from proteins such as casein, whey protein, corn zein, wheat gluten, and soy protein were reviewed by Gennadios et al. (1994a).

The film-forming ability of soy proteins has traditionally been utilized in the Far East for production of soy protein-lipid films called yuba films (Wang 1981). The process of yuba film formation consists of boiling soy milk in shallow pans; collecting the films formed (due to surface dehydration) by means of rods; and hanging the films to air-dry (Gennadios and Weller 1991, Brandenburg et al. 1993). Jaynes and Chou (1975) developed a method to prepare soy films by spreading soy protein isolate solutions on Teflon-coated baking pans followed by baking the pans at 100ºC for 1 hr. Deposition and drying of soy protein solutions is a more promising method for commercial-scale film production than the traditional yuba film process, since it allows for more consistency and control during film formation (Gennadios and Weller 1991). Gennadios et al. (1993a) described a method to develop homogeneous free-standing edible films from commercial isolated soy protein using glycerol as a plasticizer.

Soybean protein consists of two major globulin fractions, the 7S (β-conglycinin) and the 11S (glycinin), which make up 37 and 31%, respectively, of the total extractable proteins (Gennadios et al. 1994a). Thanh and Shibasaki (1976) found that the 7S and 11S fractions constituted 48 and 35%, respectively, of total protein. The 7S protein is extensively glycosylated and can assume as many as seven different forms due to combinations of its three peptide subunits a, a′, and b (Iwabuchi and Yamauchi 1987). On the other hand, the 11S protein does not contain an appreciable amount of carbohydrate, and its subunits differ in charge and molecular weight (Iwabuchi and Yamauchi 1987). These structural differences contribute to variations in the functional properties of 7S and 11S fractions (Romijn et al. 1991). For instance, the 11S fraction has a more significant impact on gelation characteristics of soy protein than does the 7S fraction (Nakamura et al. 1985). Film formation on the surface of heated aqueous solutions of 7S and 11S soy proteins, similar to the formation of yuba films on the surface of heated soy milk, has been reported (Shirai et al. 1974, Okamoto 1978). However, no information can be found in the literature related to cast films from 7S and 11S soy protein fractions.

The purpose of this study was to compare the tensile strength, elongation at break, water vapor permeability, solubility in water, and color properties of films formed from commercial soy protein isolate to those of films formed from laboratory-prepared crude 7S, crude 11S, and soy protein isolate.

MATERIALS AND METHODS

Reagents

Defatted, low-heat treated soy flakes were obtained from Archer Daniels Midland Co. (Lincoln, NE). Glycerol was purchased from Fisher Scientific Co. (Pittsburgh, PA). A food grade soy protein isolate (SUPRO 620) was obtained from Protein Technologies International (St. Louis, MO). Sodium hydroxide, hydrochloric acid, 2-mercaptoethanol, Tris-HCl buffer, sodium azide, and bicinchoninic acid (BCA) protein assay kit were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium dodecyl sulfate (SDS), acrylamide-bisacrylamide, ammonium persulphate, tetramethylethylenediamine (TEMED), bromophenol blue, and coomassie brilliant blue R-250, all of electrophoresis grade, were purchased from Fisher Scientific Co. (Pittsburgh, PA). Boric acid and sodium hydroxide required for protein determination by the Kjeldahl method were purchased from Ricca Chemical Co. (Arlington, TX), sulfuric acid was purchased from Baxter (McGraw Park, IL), and the catalyst tablets were purchased from Fisher Scientific Co. (Pittsburgh, PA).

Protein Isolation

Soy protein isolate was prepared from finely ground, low-heat treated, defatted soy flakes as described by Romijn et al. (1991). The soy globulin fractions 7S and 11S were isolated from the same source (defatted soy flakes) using a simultaneous fractionation method developed by Thanh and Shibasaki (1976). This method was based on the differential solubilities of the two globulin fractions at different pH values. Pellets of crude 7S, crude 11S, and soy protein isolate were redissolved separately in 0.03M Tris-HCl buffer at pH 7.8 (1:40 protein pellets to buffer ratio) and dialyzed overnight using Spectra/Per molecular porous dialysis tubing with a molecular weight cutoff of 6,000–8,000 Da (Spectrum Medical Industries, Inc., Los Angeles, CA). The dia-

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lyzed protein solutions were frozen and freeze-dried (stopping tray drier, model 77545, Labconco, Kansas City, MO). Temperature programming for 2,000 mL of frozen solution was –40 to 0°C with a ramp of 0.05°C/min and hold time of 10 hr for each stage. The resultant white, fluffy, and shiny protein powder was stored at –20°C until needed.

**Gel Electrophoresis**

Purities of the laboratory-prepared 7S, 11S, and soy protein isolate were assessed using a SDS-PAGE method described by Hames and Rickwood (1981). Slab gel, discontinuous SDS-PAGE was performed using a Mini-PROTEAN II electrophoresis cell (Bio-Rad Laboratories, Hercules, CA). A 10% resolving gel solution was prepared by mixing 33.3% (v/v) acrylamide-bisacrylamide (30:0.8); 12.5% (v/v) 1.5M Tris-HCl buffer (pH 8.8); 1% (v/v) of 10% SDS solution; and 4.66% (v/v) of 1.5% ammonium persulphate solution in water. The stacking gel solution was prepared by mixing 12.5% (v/v) acrylamide-bisacrylamide (30:0.8); 25% (v/v) 0.5M Tris-HCl buffer (pH 6.8); 1% (v/v) of 10% SDS solution; and 5% (v/v) of 1.5% ammonium persulphate. Both gel solutions were polymerized using 0.05% (v/v) TEMED. Protein samples were prepared by heating =3 mg of protein in 1 mL of a solution of 2% SDS, 5% 2-mercaptoethanol, 0.002% bromophenol blue, and 10% glycerol in 0.625M Tris-HCl buffer (pH 6.8). About 10 μg of each protein solution was loaded onto the gel. Electrophoresis was performed at constant 200V until the dye front reached the bottom. Gels were stained in a solution of 0.1% (w/v) coomassie brilliant blue R-250 in 50% methanol and 40% distilled water and 10% glacial acetic acid (v/v). Gels were destained using 50% methanol and 40% distilled water and 10% glacial acetic acid (v/v).

**Protein Content Determination**

Protein content of oven-dried (130°C for 4 hr) laboratory-prepared crude 7S, 11S, and soy protein isolate fractions and commercial soy protein isolate powder were determined in triplicate according to AOAC method 981.10 (AOAC 1990) using a 6.25 nitrogen conversion factor.

**Film Preparation**

Film-forming solutions were prepared by stirring commercial soy protein isolate (CSI), laboratory-prepared soy protein isolate (LSI), crude 7S, or crude 11S protein (5%, w/v) and glycerol (1.5%, w/v) in distilled water for 15 min. The pH values of the solutions were adjusted to 10.0 ± 0.1 with 2 M sodium hydroxide. Subsequently, the solutions were heated in a water bath (Magni Whirl, Blu M Electric Company, Blue Island, IL) at 85°C for 30 min, filtered through cheese cloth (grade 40, Fisher Scientific) and placed in a refrigerator at –20°C until needed.

**Water Vapor Permeability**

Water vapor permeability (WVP) of films was determined gravimetrically using methodology similar to that described by Gennadios et al (1994b). CUPS with an open mouth area of 16.6 cm² were filled with 15 mL of distilled water, and film specimens (7 × 7 cm) were sharply peaked on the cups by means of a lid, four screws, and a rubber O-ring. The cup assemblies were weighted and placed in an environmental chamber at 50% rh and 25°C. Air circulation inside the chamber was provided by a fan (198 m/min air speed). Moisture loss was monitored by weighing the cups at 1-hr intervals during an 8-hr period. Steady state was reached within 2 hr. Linear regression-derived slopes of the steady state (linear) portion of weight loss versus time curves were used to estimate water vapor transmission rate (WVTR) in g/m²/hr. WVP (g × mm/m²/hr × kPa) was calculated as: WVP = WVTR(ΔP/L), where L is film thickness (mm) and ΔP is partial water vapor pressure difference between the two sides of the film (kPa).

**Color Measurement**

Color values of soy protein films were measured with a chroma meter (CR-300, Minolta Camera Co., Osaka, Japan). Film specimens were placed on the surface of a white standard plate (calibration plate CR-AA3) and Hunter L, a, and b color values were measured. The three color coordinates ranges were: L (0 black to 100 white), a (–greenness to + redness), and b (–blueness to + yellowness).
to + yellowness) (Francis and Clydesdale 1975). Total color difference \((\Delta E)\) was calculated as:

\[
\Delta E = \left[ (L_{film} - L_{standard})^2 + (a_{film} - a_{standard})^2 + (b_{film} - b_{standard})^2 \right]^{0.5}
\]

Standard values refer to the white calibration plate \((L = 96.86, a = -0.02, \) and \(b = 1.99)\).

**Statistical Analysis**

Measurements of each property were replicated three times for each type of film, with individually prepared and cast films as the replicated experimental units. Each property replicate was the mean of two tested sampling units taken from the same film. Statistics on a completely randomized design were determined using the General Linear Models procedure in the SAS program (SAS 1988). Mean property values were separated \((P < 0.05)\) with Duncan’s multiple range test (Steel and Torrie 1980).

**RESULTS AND DISCUSSION**

**Gel Electrophoresis**

SDS-PAGE gels prepared in this study are shown in Figure 1. Both laboratory-prepared 7S and 11S fractions had relatively high purity. The level of cross-contamination between the two fractions was typical for the isolation technique used (Thanh and Shibasaki 1976, Winters et al. 1990). Similar electrophoretic patterns of protein subunits were obtained for CSI and LSI.

**Tensile Strength and Elongation**

Mean TS and E values for 7S, 11S, CSI, and LSI films are presented in Table I. Films made from 11S soy globulin fraction had significantly \((P < 0.05)\) greater TS, by \(\approx 46\%\), than did films from 7S. Similarly, it has been previously reported that yuba-like films (formed on the surface of heated soy protein solutions) from 11S were stronger than films from 7S (Shirai et al. 1974, Okamoto, 1978). Covalent disulfide bonds are believed to play a major role in soy protein film formation (Gennadios and Weller 1991). Therefore, the higher tendency of 11S protein to form disulfide bonds than 7S protein (Saio et al. 1971) could be responsible for 11S films being stronger. LSI films had greater \((P < 0.05)\) TS than did CSI films (Table I). This was attributed to LSI powder having a higher protein content (94.4%, db) than CSI powder (91.5%, db), since nonprotein constituents are likely to disrupt the homo- geneity and continuity of the protein film network. In agreement with this, Wall and Beckwith (1969) showed that commercial wheat gluten did not yield as strong a film as laboratory-prepared wheat gluten. Also, Gennadios and Weller (1992) reported that films prepared from a 75% (db) protein content commercial wheat gluten had lower TS than that of films prepared from an 82% (db) protein content commercial wheat gluten.

In contrast to TS, no significant \((P > 0.05)\) differences were detected among mean E values of 7S, 11S, CSI, and LSI films (Table I). It should be noted that film thickness was not accounted for in calculation of E values as was the case with calculation of TS values. Therefore, the substantial variability in measured E values and failure to detect differences among mean values may have been due to the small thickness variations of tensile testing film specimens.

**Solubility in Water**

Stuchell and Krochta (1994) reported that pieces of glycerol-plasticized films from commercial soy protein isolate maintained their integrity after incubation in water for 24 hr with occasional gentle agitation. They suggested that only monomers, small peptides, and nonprotein materials solubilized in water. Film pieces immersed in water in the present study were not broken apart and the film network remained intact. Most likely, the hydrophilic glycerol constituted a large part of the film TSM.

**TABLE I**

<table>
<thead>
<tr>
<th>Film</th>
<th>TS (MPa)</th>
<th>E (%)</th>
<th>PS (%)</th>
<th>TSM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7S</td>
<td>7.36 ± 0.42a</td>
<td>37.06 ± 18.5a</td>
<td>8.59 ± 0.20c</td>
<td>39.41 ± 1.56d</td>
</tr>
<tr>
<td>11S</td>
<td>10.77 ± 0.63b</td>
<td>37.93 ± 18.5a</td>
<td>3.99 ± 0.05a</td>
<td>26.81 ± 0.16a</td>
</tr>
<tr>
<td>CSI</td>
<td>8.53 ± 0.48a</td>
<td>31.93 ± 2.4a</td>
<td>6.51 ± 0.26b</td>
<td>35.11 ± 0.99c</td>
</tr>
<tr>
<td>LSI</td>
<td>10.68 ± 0.95b</td>
<td>41.66 ± 2.2a</td>
<td>12.19 ± 0.30d</td>
<td>29.91 ± 1.95b</td>
</tr>
</tbody>
</table>

a Means of three replicates ± a standard deviation. Any two means in the same column followed by the same letter are not significantly \((P > 0.05)\) different according to Duncan’s multiple range test.

b Commercial soy protein isolate.

c Laboratory-prepared soy protein isolate.

d Laboratory-prepared soy protein isolate.

**TABLE II**

<table>
<thead>
<tr>
<th>Film</th>
<th>Thickness (µm)</th>
<th>WVP (g/mm2/m/hr/kPa)</th>
<th>rh (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7S</td>
<td>65 ± 4.5</td>
<td>4.39 ± 0.59</td>
<td>73.14 ± 0.76</td>
</tr>
<tr>
<td>11S</td>
<td>57 ± 7.0</td>
<td>4.27 ± 0.54</td>
<td>71.82 ± 0.16</td>
</tr>
<tr>
<td>CSI</td>
<td>54 ± 6.0</td>
<td>3.76 ± 0.16</td>
<td>73.26 ± 1.20</td>
</tr>
<tr>
<td>LSI</td>
<td>62 ± 6.1</td>
<td>3.88 ± 0.30</td>
<td>74.00 ± 0.53</td>
</tr>
</tbody>
</table>

a Means of three replicates ± a standard deviation. No significant \((P > 0.05)\) differences were detected among WVP means.

b Actual rh values at the underside of films calculated as described by Gennadios et al. (1994)b to account for resistance of stagnant air layer between film and water surface in testing cups. rh outside the cups was 50%.

c Commercial soy protein isolate.

d Laboratory-prepared soy protein isolate.

**TABLE III**

<table>
<thead>
<tr>
<th>Film</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>ΔE</th>
</tr>
</thead>
<tbody>
<tr>
<td>7S</td>
<td>94.25 ± 0.35a</td>
<td>-2.42 ± 0.25a</td>
<td>11.64 ± 1.05ab</td>
<td>10.28 ± 1.12ab</td>
</tr>
<tr>
<td>11S</td>
<td>94.83 ± 0.44a</td>
<td>-3.16 ± 0.16b</td>
<td>10.66 ± 0.70b</td>
<td>9.55 ± 0.68b</td>
</tr>
<tr>
<td>CSI</td>
<td>93.05 ± 0.40b</td>
<td>-2.46 ± 0.34a</td>
<td>12.62 ± 1.43a</td>
<td>11.56 ± 1.50a</td>
</tr>
<tr>
<td>LSI</td>
<td>94.77 ± 0.16a</td>
<td>-2.30 ± 0.18a</td>
<td>9.93 ± 0.87b</td>
<td>8.53 ± 0.79b</td>
</tr>
</tbody>
</table>

a Film color was measured by placing films on top of a standard white plate \((L = 96.86, a = -0.02, \) and \(b = 1.99)\). Presented values are means of three replicates ± a standard deviation. Any two means in the same column followed by the same letter are not significantly \((P > 0.05)\) different according to Duncan’s multiple range test.

b Commercial soy protein isolate.

c Laboratory-prepared soy protein isolate.

d Laboratory-prepared soy protein isolate.
Films from 7S had higher ($P < 0.05$) TSM and PS values than 11S films (Table I). This was attributed to the lower molecular weight of 7S proteins, the higher tendency of 11S proteins to form disulfide bonds (Saio et al 1971), which stabilize the film network, and the more lipophilic nature of 11S proteins (Watanabe et al 1975). The higher ($P < 0.05$) TSM of CSI films compared to LSI films (Table I) was likely due to the presence of a greater amount of nonprotein impurities in the CSI powder. However, CSI films had lower ($P < 0.05$) PS than did LSI films.

**Water Vapor Permeability**

No significant ($P > 0.05$) differences were detected among WVP values of 7S, 11S, CSI, and LSI films (Table II). In general, protein films are poor water vapor barriers due to the inherent high hydrophilicity of proteins and the substantial amount of hydrophilic plasticizers added to protein films (Gennadios et al 1994a). For the sake of comparison, low density polyethylene films have WVP values (25 μm films, 90% rh gradient, 38°C) ranging from 0.0026 to 0.0035 g × mm/m² × hr × kPa (Briston 1988), which are lower by three orders of magnitude than WVP values of soy protein films measured in the present study.

**Film Color**

Color is an important property of protein films because it could affect consumer acceptance of such films in potential edible or nonedible packaging applications. Hunter L, a, and b color values and total color differences for 7S, 11S, CSI, and LSI films are shown in Table III. CSI films were slightly but significantly ($P < 0.05$) darker (lower mean L value) and more yellow (greater mean positive b value) than the other three types of films. This was attributed to the greater amount of impurities present in the CSI powder.

**Implications and Potential Applications**

This study showed that soy protein isolate of increased purity can yield stronger films than commercially available soy protein isolate. Also, use of 11S soy globulin fraction alone can give stronger films than commercial soy protein isolate. However, this improvement in film strength would come at the additional costs of further purifying soy protein isolate or separating the 11S protein fraction. Although soy protein films are poor water vapor barriers, they have been found to be very effective oxygen barriers (Brandenburg et al 1993, Gennadios et al 1993b). The good oxygen barrier ability of soy protein films could be utilized in the manufacture of multilayer packagings where soy protein films would function as the oxygen barrier-providing layer. In another manufacture of multilayer packagings where soy protein films (Brandenburg et al 1993, Gennadios et al 1993b). The good oxygen-barriers, they have been found to be very effective oxygen barriers of further purifying soy protein isolate or separating the 11S protein to the formation and properties of yuba-film. Nippon Shokuhin Kogyo Gakkaishi 21:324-328.

**REFERENCES**

References are cited in the text. A complete list of references can be found at the end of the manuscript. This research was partially funded by the Nebraska Soybean Development, Utilization, and Marketing Board.

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**LITERATURE CITED**

